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STUDY OF A POSSIBLE RELATIONSHIP BETWEEN BILHARZIASIS AND NEPHR--ETC(U)
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(6) Study of a possible relationship between Bilharziasis and Nephrotic Syndrome in Upper Egypt.

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As stated elsewhere there exists a highly suspected correlation between shistosomiasis and Glomerulonephritis (Nephrotic Syndrome).

Long lasting clinical observation of such a possible relationship has lead us on 1972 to start a five year study for such a possibility.

Both Epidemiological and immunological data collected over the last three years strongly validated the hyposthesis of a cause and effect relationship between Shistosomiasis and Glomerulonephritis.

The steps of work performed over the last year were same as previous years. We were aiming at collecting more data to proove or invalidate previous conclusions.

The epidemiologic study pursued the same road as that in the preceeding two years. Three more villages have been surveyed, thus making a total of sixteen studies in fourteen villages. The last three villages had variable duration of perennial irrigation. Table I shows the number of population studied in every village and the period of time elapsed since the introduction of Nile water.

As shown in the table the percentage of the population studied varied

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from 43.08 to 71.96, it was dependent upon the size of the village and availability of men during our working hours. E.M. village has been previously studied at periods 0 and 8 months after irrigation. The prevalance of Schistosomiasis and Proteinuria in the last examined three villages are shown in Table II. As disclosed from the table the percentage of Schistosomiasis was highest after 12 years of irrigation than shortly (18 months) or long (16 years) after water introduction.

The same finding was obtained in our previous work when we found that the percentage of infected individuals in various villages increased with longer duration of irrigation till 12 years (71.6) and then dropped to 52% in a village having Nile water for 38 years. In E.M. Schistosomiasis affected 5.6%, 46.16% and 47% of the population studied at 0, 8 months and 18 months of irrigation. Last survey was done during winter time where the boys are in school, they do not bathe in the canal, there is not much work in the fields and usually at that time of the year the Sh. snails are hidden in the mud and cercaria (the infecting stage of the parasite) does not survive in the cold water.

The table also shows that the percentage of heavy proteinuria is higher in E.M. rather than the other two villages. Parallal results were obtained in the previous two years when it was found that the prevalence of heavy proteinuria increased steeply as a function of time after irrigation till the middle of the second year (9.9%) and then dropped to 2-5% at the following years. In a village examined twelve years following irrigation heavy proteinuria was found affecting 1.9% of the population.

In E.M. the percentage of heavy proteinuria climbed up from 0.001 to 2.92 and then to 4.23 at periods 0, 8 & 18 months after introduction of canal irrigation. The rise of the prevalence of proteinuria surpassed during that period, the increase in the prevalence of Schistosomiasis. Such proteinuria was virtually confined to young subjects (less than 30 years) in N.S. (16 years of Nile water) and Z.W. (12 years) while it was affecting other age groups in E.M. though still predominant in younger population (Table III).

Moderate proteinuria was found more commonly in chronically irrigated villages. In most cases pus cells were also found.

Urinary casts and renal epithelial cells were more commonly found in E.M.

When comparing the prevalence of possible immunologic diseases in various villages we got the following (Table IV).

1. Skin rashes in form of urticaria, marked itching and impetigo is clearly more common in Bilharzial than in non Bilharzial patients in villages recently irrigated while no difference was noted in these having canals for more than 12 years. In E.M. skin rash were more common in non-Bilharzial cases before irrigation while the number increased up to 90 and 62 cases at 8 & 18 months after irrigation. The rash was transient and some of them could date it as occuring 15 to 30 days before dysuria & haematuria.

2. Short lasting rheumatoid-like arthritis was common in Bilharzial patients with more prevalence among residents of the chronically irrigated villages.

Arthritis was found in association of urinary symptoms and in some with heavy proteinuria.

As an attempt to throw some light on the individual susceptibility to acquire immunologic diseases (e.g. Glomerulonephritis) the capacity of the body to eliminate particulate matter has been tested for in ninety six individuals by the P.V.P. clearance test. (The work was performed with the collaboration of Prof. J. SOOTHILL, London). This test assumes that people with low P.V.P. clearance are not capable of immune complexes disposition and hence complexes will circulate to be deposited in the wall of the blood vessels and glomerular capillaries initiating tissue injury. It has been found to correlate well with tests done for measuring antibody kinetics. Those 69 individuals were free from Schistosomiasis when their clearance was measured (February 1975).

They were then reexamined at different intervals for evidence of Schistosomiasis and heavy proteinuria. In June 1975 twelve of them developped schistosomiasis and five had heavy proteinuria: four of those had very low P.V.P. clearance while the fifth was the best to clear that substance. They were all treated with Niridazole. During the next August retesting for schistosomiasis and heavy proteinuria revealed positive ova in seventeen cases. Five of the latter had been passing ova in the preceeding June. Heavy proteinuria and casts were detected in nine instances. Proteinuria was newly discovered in eight cases and persistent since June only in one. All patients (except one) with proteinuria on June had urine free of protein while two of them were still passing ova. Two patients with positive schistosomiasis on June and August got proteinuria on August.

The same study was repeated in November 1975 & March 1976. In November nine were still passing ova in the urine and new six cases were discovered. Only one patient had persistent proteinuria and nine fresh cases were found. One of the latter had negative tests for schistosomiasis after being treated in August.

Study in March showed persistence of Sh. in seven, Sh. and proteinuria in five, fresh schistosomiasis in seven and proteinuria in one.

P.V.P. clearance values will be checked to assess whether those developing proteinuria would have any special pattern. In addition complement study (C_{1g}, C_{2y} opsonization) has been also performed on the entire group. Determination of Sh. worm antigen will be done for positive cases.

Patients: As was previously performed every nephrotic patient admitted to the ward has been checked together with his healthy control for Sh. ova and heavy proteinuria. Immunoelectrophoresis for C_3 - C_{3d} degradation was also studied for both patient and control. From 80 nephrotics admitted since May 75 46 had schistosomiasis with or without urinary tract abnormality and other associated infections. The 74 controls obtained for matching were all free from schistosomiasis except four. Addition of those figures to the previously obtained ones will make an overall result shown in Table V,

	Controls	Patients
Sh. -	240	114
Sh +ve	34	216
Total	274	330

C_{3d} was demonstrated in 68 nephrotics and in two controls

Clinical and Immunological Studies

Patient Material

Males aged from 10 to 50 years, fulfilling the criteria of Nephrotic Syndrome and residing in Assiut Province were studied.

Methods of studies:

Same detailed clinical, biochemical, immunochemical and immunopathological investigations have been performed as has been previously described. More detailed study on complement was added this year as well as testing for the Nephrogenic factor in patients serum.

Results:

80 patients with Nephrotic Syndrome have been studied over the past year. From those sixteen were fulfilling the criteria considered to characterize Schistosoma Associated Glomerulonephritis.

Clinical characteristics:

Twelve patients were in their second or third decade of life (from 15 to 25 years old). The other four were of age 32 - 38 years. They were all village residents. History of schistosomiasis was only manifested as mild short-lasting dysuria & haematuria. In five of them ova were found in the urine while in the rest they were only detected in the rectal smear.

Intermittent attacks of fever, skin rash and arthritis were common. Brochospasm was found in nine of them. Oedema was acute in onset in eleven and insiduous in five. Ascites was found in four.

B.P. was normal in 14 and slightly raised in two. Hepatomegaly varying

from soft tumour to a quite firm cirrhotic liver was detected in twelve cases. Splenomegaly was found in eight cases.

Blood chemistry:

Serum proteins were ranging from 3 to 7.6 g/100 mls. Albumin was universally less than 2.5g (0.5 - 2). Alpha - 2 globulin was raised in four and normal in the rest. Gamma globulins were raised in fourteen cases.

B.U.N. was within the normal range in 13 cases and 60, 72 and 80 in the last three. S. creatinine was less than 1.5 mg /100 ml in fourteen and 2, 2.5 in the remaining two. Creatinine clearance ranged from 75 to 120 ml/n in twelve, from 32 to 70 in three and 22 mls in one case.

Serum Ig G was ranging from 800 to 2000 mg/100 ml, S. Ig M from 80 to 200, S. Ig A 100 to 400 and S. Ig E was more than 2000 units and highest value was recorded in two patients with history of quite recent bilharziasis and soft hepatic enlargement.

Urine albumin was more than 6g/24 hours and their permeability index ~~was~~ ranged from 0.004 to 0.09.

Urine excretion of immunoglobulins was as follows:
Ig G : 400 - 1000 mg /100 mls and Ig M 3 - 30 mg /100 mls. The differential protein clearance was less than 0.2 in two case and more than that ratio in the fourteen.

Urine Ig G and Ig M were found in high concentration when antiworm antibodies were demonstrated in the urine.

The titre of Rheumatoid factor was more than 64 in all. It ~~was~~ ranged from 256 to 320 in six cases with recent onset of Nephritis and liver cirrhosis

and 320 units in other two cases having circulating antiworm antibodies.

Circulating worm antigen were detected in ten cases while circulating antiworm antibodies were found in eight instances. The latter were having the highest level of Ig Gn Ig M.

Complement profile:

CH50 was less than 20 units in all.

C3 was ranged between 20 and 50 mg/100ml in ten and was more than 80 mgs in three.

C4 was ranged from 8 to 12 in 13 and 18-20 in the other three.

C3PA was normal in four and less than 50% N in the rest. As has been previously noted, low complement activity was noted when circulating Ag and Abs were detected. Table VI shows the values of CH50 and other complement components in all the patients studied over the previous three years when grouped according to whether they have or have not circulating antigens and antibodies.

Nephrogenic factor was detected in 12 cases from the sixteen studied last year. Thus making 34 position cases among 46 determinations in the whole work. That factor is now considered to be an important mediator for the perpetuation of alternate pathway activation and persistence of glomerular damage due to immunological reactions.

Repeated testing for complement after disappearance of oedema and proteinuria showed resumption to normal in twelve while CH50 and C3 remained low in four. Those last patients were showing persistence of Ag or Ab in their sera.

Kidney Morphological Changes:

Kidny biopsy was done as previously described and a successful renal cortex was obtained in fourteen cases. Histopathologic pictures were consistent with the same type found in 90 biopsy specimens examined over the last three year. All were falling within one or other variety of Mesangio-capillary Glomerulonephritis with varrying degrees of mesangial stalk changes, hypercellularity and thickening of the capillary walls.

A. Five of the cases showed changes considered as type IIA in the previous reports: Glomerular swelling, proliferation of the tuft by mesangial and endothelial cells, obliteration of the tuft - capsular space, thickening of the mesangial fibres and patchy thickening of the capillary wall. The polar vessels were thickened by subintimal proliferation in three instances. Periglomerular cellular infiltration was found in two. Tubules were more or less normal but contained variety of casts: hyaline, cellular blood. The age of the patients was ranging from 15 to 25 years. They all were having short history of oedema and in three the liver was enlarged. Rheumatoid like arthritis was manifest in three.

B. Seven biopsy specimens disclosed the changes previously described as IIB abnormalities: The size of the glomerulus was increased and same changes described in IIA were there except that cellularity was less and capillary wall thickening was uniform with some splitting. Vascular and interstitial changes were the same as in IIA.

In both A . B Masson green subendothelial deposits were common.

The age of patients was nearly the same as IIA but the duration of nephritis was longer. Liver fibrosis was more advanced in those cases.

C. Two cases showed changes considered as type III Mesangiocapillary Glomerulonephritis: The size of the glomerulus was either enlarged or normal, cellular proliferation existed to a lesser extent but characteristically epithelial proliferation was quite distinct with tuft, capsular adhesions, crescents formation and capsular thickening or fibrosis. Tuft lobulation was much exaggerated and thickening of the capillary wall was more apparent. Vascular changes were apparent and in one proximal tubules showed ischemic changes. The two were aged more than 30 years. Both had marked liver fibrosis with liver shrinkage in one.

Table VII shows the distribution of various morphological types among the age groups of the 104 biopsy specimens examined over the three years study period.

Immunopathologic study:

Snap frozen biopsy specimens were stained by F.I.T. conjugated monospecific antisera: anti IgG, M, A & E anti C_3, C_4, C3PA, antifibrinogen, antialbumin and antischistosoma worm, antistreptolysin O, anti E-coli and antiserum to Malaria (Detailed method has been described in the Second Annual Report).

From sixteen tests performed on 16 specimens the results were:

1. Ig G deposits were found in twelve specimens: in four it was localized to the glomerulus while in the rest it deposits were found in the glomerulus, small vessels and proximal tubules. Deposits were fine grassular and mostly distributed on the peripheral loops. The corresponding morphology was consistent with type IIB and III in all except three.

2. Ig M was positive in four biopsies. Deposits were confined to the glomerulus in two cases and affecting both glomerulus and polar vessels in other two. Deposits were coarsly granular and more centrilobular. The morphology of those four specimens was that of type II A with cellular proliferation and minimal capillary changes. The duration of nephritis was rather short. They had lowest value for CH50, C3 & C4.

3. Ig A was deposited in 4 cases. Deposits were discrete star like and found mainly in the central loops of the glomerulus. They were associated with type II B (one) and type III pathological changes. C3PA level was characteristically lowered in those three cases.

4. Ig E was deposited in 3 cases as discrete coarse granules.

5. Complement factor C3 was deposited in all the specimens: it was involving the glomerulus only in three while appearing in the vessels wall and the tubules in the rest. Deposits were granular distributed in a segmental or diffuse pattern in the mesangium and along the capillary walls staining was denser in specimens of cases passing antiworm antibodies in the urine. In two specimens C3 was found with total abscence of Igs deposits.

C4 deposits were found in fourteen out from the sixteen cases examined. Deposits were fine granular, found along the capillary wall and restricted to the glomerulus. They were accompanied by Ig G deposits.

C3PA was positive in thirteen specimens. In three cases there was no Ig deposits. Tubu'ar deposits were fine granular and mostly in the cytoplasm. Vessels were negative.

6. Fibrinogen deposits were found in three cases. Deposits were peripheral and the corresponding pathology was type III.

7. Schistosoma worm antigen was found in ten cases. Glomeruli only were involved in six. Deposits were coarse and fine granular, discrete, either focal or diffuse. Corresponding morphology was Type II A & II B. Circulating antigen was found in four and Abs in 2.

Stainings for streptolysin, E. Coli, H.S.A. and Malaria were negative. Table VIII shows collective data obtained since the start of the work.

Elution Studies:

Elution with citric acid buffer (p.H. 2.5 - 2.8) was done on six specimens with positive Sh. worms antigen. The supernatant was studied after concentration for Sh. Ag - Ab by C.E.P. technique. Specific antiworm antibodies were detected in two cases and Sh. worm antigen in four specimens. Restaining of of the sections after elution did not reveal any deposit.

Same clinical trials conducted during the last three years were continued to study the response of that type of Glomerulonephritis to therapy: Niridazole, Corticoids and Cyclophosphamide. Study on the last sixteen patient revealed:-

1) Early remission with Niridazole, in ten cases. Two of those developped a transit relapse six months later on and the rest are still normal.

2) Four had permanent remission after corticoids.

3) Two developped two relapses and failed to respond to neither Corticoids nor Cyclophorphamide.

Conclusion:

1. Results obtained during last year study strongly solidify the hypothesis of a cause and effect relationship between schistosomiasis and Mesangiocapillary Glomerulonephritis.

2. Expansion of the epidemiological study is needed for villages with various dates of irrigation in order to obtain a curve of a possible time.

Prevalence relationship for both diseases.

3. Prospective study of E.M. during the last 1.5 years was one of clear evidences for the development of heavy proteinuria following schistosomiasis. Further longitudinal study is required for that village. A yearly survey is planned.

4. P.V.P. clearance test is of possible help in the prediction of individuals more prone to develop immunological diseases. The 69 individuals in whom P.V.P. clearance was determined are to be followed and examined every 6 month. An Attempt is to be made to assess whether patients developing Glomerulonephritis with the exposure to a certain Ag (Schistosomiasis) are deficient in disposing immunocomplexes. Correlation between the proneness to develop Nephritis with the complement make up will also be studied.

5. Clinico immunological studies confirmed the high suspicious of the existence of a Schistosoma Associated Glomerulonephritis. Elution studies advanced solid evidences. Future Planning Includes:

a. Expansion of the elution tests.

b. Trial to demonstrate circulating immuno complexes.

c. Immunofluorescent demonstration of individual worm antigens (? surface) using antisera to purified fractions.

d. Trial to biopsy patients having heavy proteinuria and casts in the affected village, thus describing early transit lesions.

References

1. Contract No N00014-73-C-0007. Second Annual Report, April 1975.

2. A.G. Morgan, J.F. Soothill" Relationship between macrophage clearance of P.V.P. and affinity of anti-protein antibody response in inbred mouse. Nature, 254: 711, 1975.

Table I

Name of Village	Duration of irrigation in years	Total of population	Population Studied	
			Number	% of total
(1) Z. W.	12	2600	1166	43.08
(2) N. S.	16	2000	1283	64.15
(3) E. M.	1.5	1320	945	71.9

The number of population studied in three villages with various periods of perennial irrigation.

Table II

Name of village	Duration of Perennial Irrigation	Population studied	Schistosomiasis		Proteinuria			
			No	%	Moderate		Heavy	
					No	%	No	%
Z. W.	12	1166	795	68	24	2.05	16	.3
N. S.	16	1283	706	57	68	5.3	22	.7
E. M.	0	1125	62	5.6	0	0	1	0.001
E. M.	8/12	1094	505	46.16	0	0	32	2.92
E.M.	1.5 Yr	945	445	47	5	0.022	40	4.23

Prevelance of Schistosomiasis (Sh.) and Proteinuria in three villages examined during the year 1975 - 1976. Included as well are the results of E.M. of 1971 & 1974.

Table III

	Z.W. 12 years No.	N.S. 16 years No.	E.M. 0 No.	E.M. 3/12 Mos. No.	E.M. 1.5 Yr. Nos
Population studied	1166	1283	1113	1094	945
Age 10 - 19 ys. Sh.	562	529	36	324	267
Prot.	20	14	0	12	25
Age 20 - 29 ys Sh.	111	94	36	83	81
Prot.	16	8	0	6	5
Age 30 - 39 ys. Sh.	77	52	0	52	55
Prot.	3	0	0	10	8
Age 40 - 50 ys. Sh.	45	21	0	46	42
Prot.	1	0	0	4	2

Prevelance of Schistosomiasis and Heavy Proteinuria among age groups on the three villages studied last year. E.M.'s figures reported on 1971 - 1974 are included for comparison.

Table IV

Name of village	Duration of irrigation ys	Total population studied	No. of Sh. patients	No of patients with skin rash		No of patients with arthritis	
				With Sh.	No Sh.	With Sh	No Sh
1 KEN	38	1351	695	1	1	1	8
2 NS (N.S.)	16	1283	706	36	0	66	2
3 K.A.S.	12	1115	763	16	5	58	8
4 Z.W.	11	1166	795	36	6	38	16
5 EL MATYAA	7	1002	517	48	5	96	49
6 B. GH.	5	629	332	3	0	4	0
7 El-Oder	4	429	218	1	4	81	109
8 MASRAA	3.5	732	313	18	2	10	2
9 BOSRA	3	549	339	36	6	38	16
10 G.E.T.	2.5	253	102	4	1	10	1
11 B.M.	2	70	43	-	-	2	1
12 G.B.	6/12	1170	329	23	4	58	5
13 El-Shanabla		250	55	-	-	4	1
14 Kom E.M.		234	127	30	3	10	1
15a E.M.	0	1133	70	10	5	30	10
b E.M.	8/12 Mas	1094	505	90	10	65	15
c E.M.	1.5	945	445	62	12	42	10

Prevelance of skin rash and transit arthritis in the population of all the villages studied from 1971 up till now.

Sh. Schistosomiasis

Table VI

Changes in the level of CH50 and concentration of various complement in patients with Sh. Associated Glomerulonephritis when grouped into those with positive and negative circulating antigen and antibodies

Test	Worm Antigen and antiworm Antibodies determined 66 patients				Antibodies only tested for 40		Test not done 40
	+ve Ag -ve Ab 10/66	+ve Ag +ve Ab 25/66	-ve Ag +ve Ab 13/66	-ve Ag -ve Ab	+ve 20/40	-ve 20/40	
units range CH50	12 - 30	13 - 34	17 - 25	23 - 34	17 - 23	22 - 27	24 - 120
less than 20	9	21	10	-	15	-	16
range C3 mg	20 - 22	20 - 36	20 - 28	28 - 90	20 - 34	27 - 52	40 - 150
less than 30	8	20	13	4	15	5	-
RANGE C3 mg	9 - 12 7/36	8 - 11 15/36	10 - 12 8/36	10 - 20 6/36	10 - 22 12/20	17 - 22 8/20	8 - 12
less than 12	6	16	8	3	7	4	8
range C3PA%N	30 - 100 5/16	20 - 92 5/16	35 - 80 3/16	28 - 90 3/16	20 -100 7/7	50 - 100 0/7	20 - 100%N
less than 50%	4	3	2	3	2	0	3

Table VII

Morphological Distribution among age groups

Type	10 - 19 years	20 - 29 ys	30 - 39 ys	40 or more	Total
I	11/40	3/44	1/15	-	15
II a	10/40	10/44	5/15	1/3	26
II b	15	21	4	-	40
III	4	9	4	2	19
IV	-	1	3	1	5
Total	40	44	17	4	105

Table VIII

Summary of Immunopathological Profile

1. Igs. and Fibrinogen

Staining	No of Tests	G + T.+ V.	G	G + V	G + T	T	Pattern
G	90	50	12	13	8	5	Fine granular Diffuse
M	90	30	18	10	2	4	Coarse granular
A	90	8	13	1	3	-	Dispersed granular
E	42	-	3	2	-	-	Granular
Fibrinogen	77	3	32	13	5		Fine granular
Albumin	60	3	-	-	4	12	Fine granular

Staining	No of tests performed	G + T + V	G	T	G + V	G + T	Pattern
C3	90	48	30		14	12	focal-coarse granular
C4	46	-	26		-	-	fine granular
C3PA	40	-	11		-	12	granular focal
Sh. Worm Antigen	60	8	14		2	6	fine & eparse granular

2. Complement components and Sh. worm antigen deposits

Figures

Figure 1 Type II A morphological type: Glomerular size is enlarged, much mesangial and endothelial cellular proliferation. Irregular thickening of the tuft. H.X X320.

Figure 2 Type B changes: Size of the glomerular is enlarged, cellular proliferation is less than in II A while capillary wall thickening is more apparent. Silver Methanamine stain X320.

Figure 3 Type 3 changes: Marked lobulation of the tuft with epithelial proliferation and adhesion to the capsule.

Figure 4 Ig G deposits in the glomerular tuft and capsule. X320. Small arteries and proximal tubules are positive.

Figure 5 Ig M deposits localised to the tuft, more concentrated in the central mesangial region.

Figure 6 Ig A deposits in the tuft: focal coarse granular and dispersed X320.

Figure 7. C3 deposits in the tuft and capsule: focal coarse granules of variable intensities X320.

Figure 8. C4 deposits in the glomerular tuft: granules are deposited along the capillary wall.

Figure 9. C3PA deposits in the tuft: coarse granules in the mesangium and capillary wall.

Figure 10. Fibrinogen deposits in a glomerular tuft and capsule X320.

Figure 11. Schistosoma worm antigen deposited in the mesangium and capillary wall of a glomerulus: granules of variable sizes.

Fig. 1.

Fig. 2.

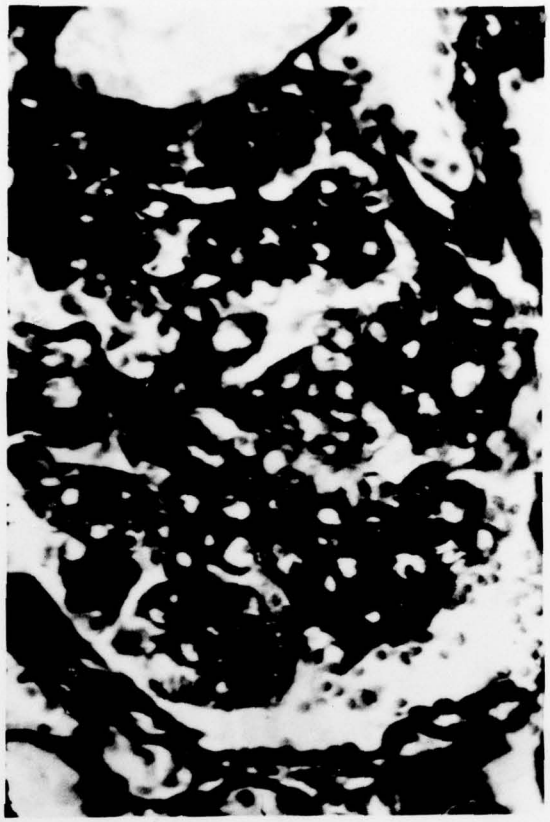

Fig. 3.

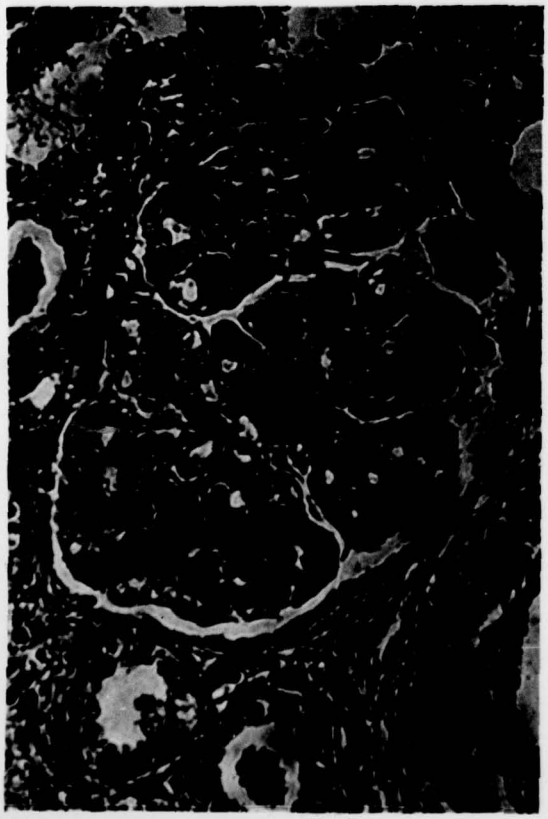

Fig. 4

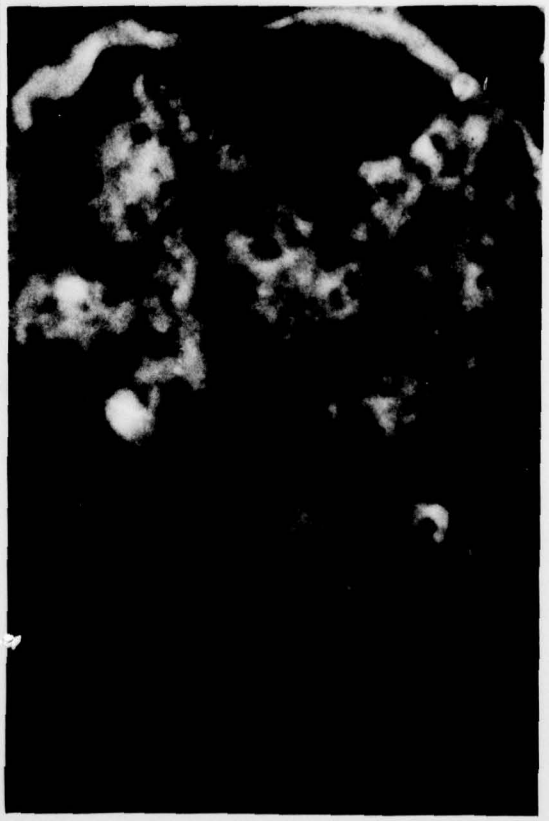

Fig. 5.

Fig. 6

Fig. 7.

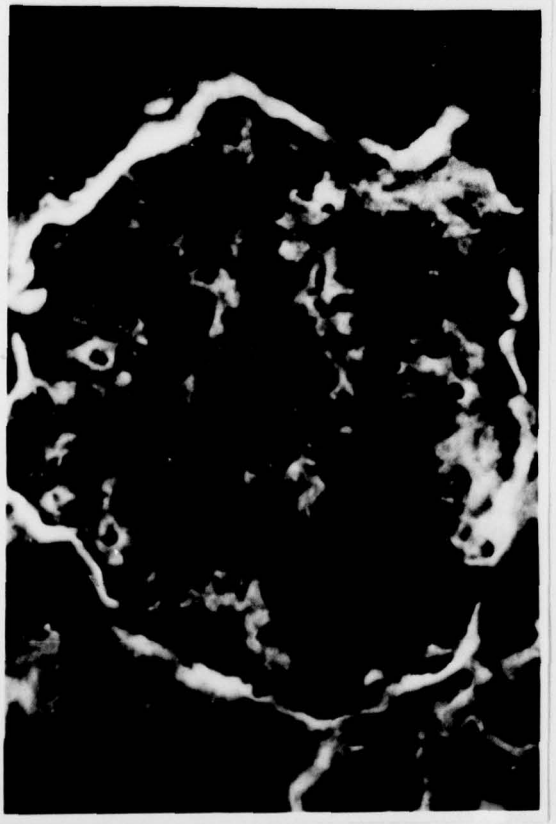

Fig. 8

Fig. 9.

Fig. 10

Fig. 11.